



MHSRS-26-4190: Novel Cell-Mediated RNA-Based Antimicrobial Solutions to Prevent Combat Wound Infections and Suited for Austere Conditions

Dr. Yoann Le Breton, Ph.D.

Combat Wound Infections Division | MRBD | NMRC

READINESS THROUGH RESEARCH & DEVELOPMENT



MHSRS-26-4190: Novel Cell-Mediated RNA-Based Antimicrobial Solutions to Prevent Combat Wound Infections and Suited for Austere Conditions

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Protocols using animals were reviewed and approved by the WRAIR/NMRC Institutional Animal Care and Use Committees in compliance with all applicable federal regulations governing the protection of animals and research.

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Conflicts of Interest



Patent Application No.: US19/742,500

Title: Antimicrobial compositions and methods

Filing date: 07/15/2026

Inventors: Yoann Le Breton (NMRC) and Evelina Angov (WRAIR)

Patent Application No.: PCT/US26/36792

Title: Antimicrobial compositions and methods

Filing date: 07/15/2026

Inventors: Yoann Le Breton (NMRC) and Evelina Angov (WRAIR)

Patent Application No.: 64/042,035

Title: Antimicrobial compositions and methods

Filing date: 04/17/2026

Inventors: Yoann Le Breton (NMRC) & Evelina Angov (WRAIR)



Combat Wound Infections Division (CWID)

Conduct translational research to develop and evaluate [antimicrobial countermeasures](#) to mitigate complications from combat wound infections; require FDA approval



dvidshub.net

Dr. Zachary Rivas: MHSRS-26-6075 (talk): Advancement of Pathogen-Agnostic CLAROMER® Peptoids as Deployable Countermeasures for Battlefield Wound Infection Treatment.

Dr. Corley Bernstein: MHSRS-26-3702 (poster): Illuminating *Klebsiella pneumoniae* Infections: A Bioluminescent Platform for Longitudinal Assessment of Antibacterial Efficacy

Monoclonal Antibodies

Dr. Lucas Brown: MHSRS-26-5513 (poster): Evaluation of Field Deployable Broadly Neutralizing Monoclonal Antibodies to Counter MRSA Infections in Combat Wounds.

Bacteriophages

Anti-Biofilm Agents

Dr. John Vitucci: MHSRS-26-3821 (poster): Efficacy of a Novel Antimicrobial Peptide in Eradicating Multidrug-Resistant Bacterial Infections and Biofilms in Preclinical Mouse and Porcine Models.

Antimicrobial Proteins/Peptides

Enhanced Dressings

mRNA Medicine

Dr. Mackenzie Ryan: MHSRS-26-4729 (poster): Engineering Antimicrobial RNA Therapeutics to Combat *Staphylococcus aureus* in Human Cell Models of Combat Wound Infections.

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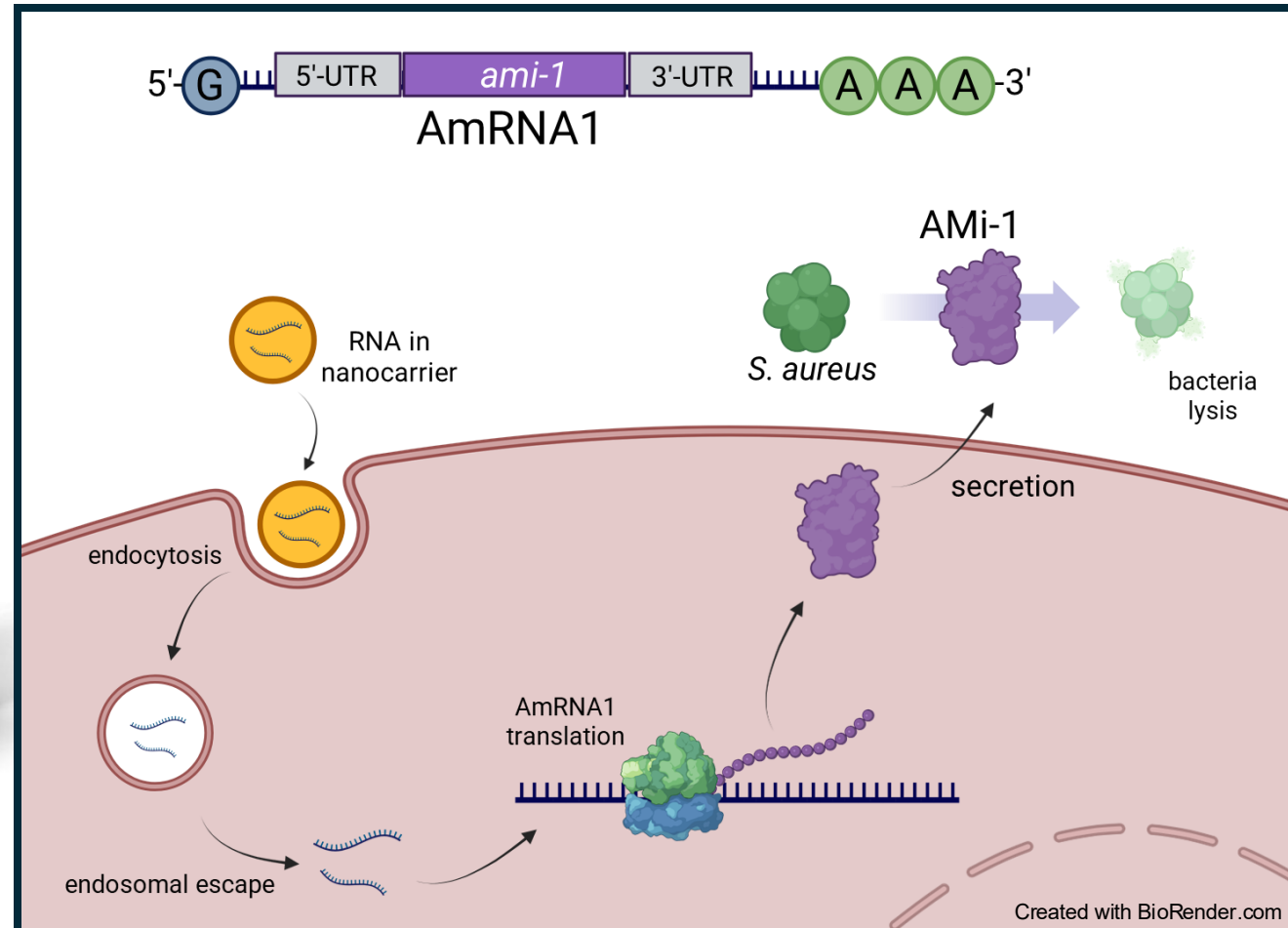
READINESS THROUGH RESEARCH & DEVELOPMENT



RNA-Based Antimicrobials



New antimicrobial product: The mRNA delivery platform used to turn our own cells into “mini-factories” transiently producing antimicrobial compounds



pharmaceuticalonline.com



freepik.com

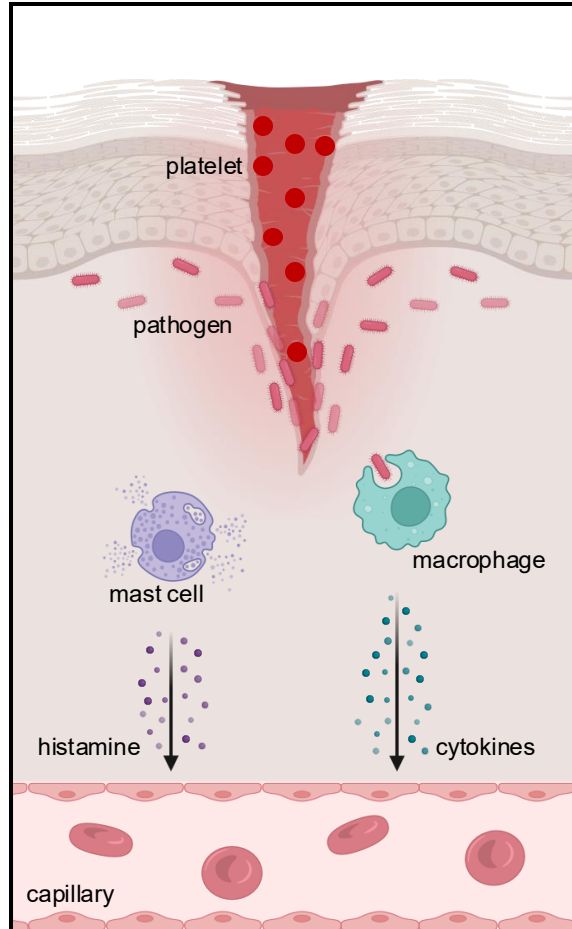
READINESS THROUGH RESEARCH & DEVELOPMENT



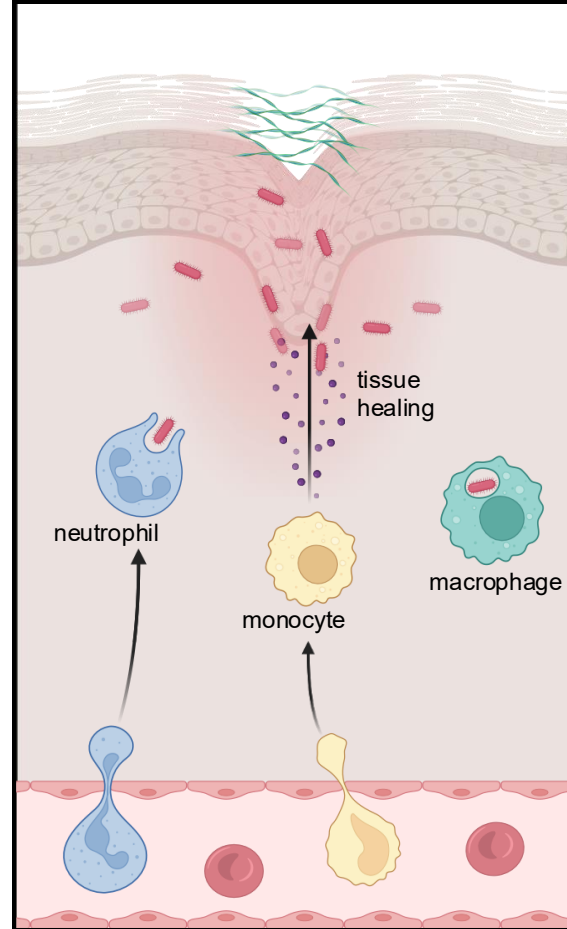
Models: Skin and Soft Tissue Wound



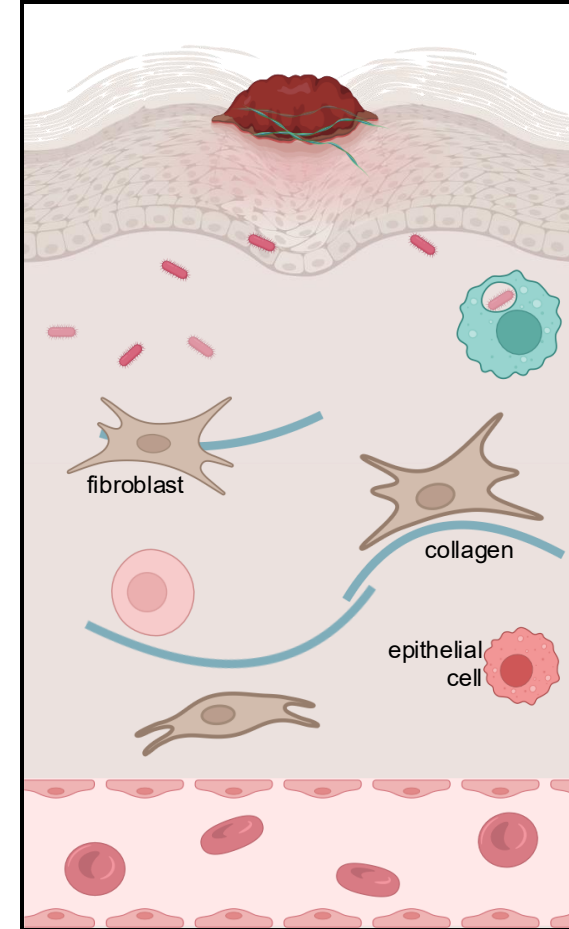
1. Hemostasis



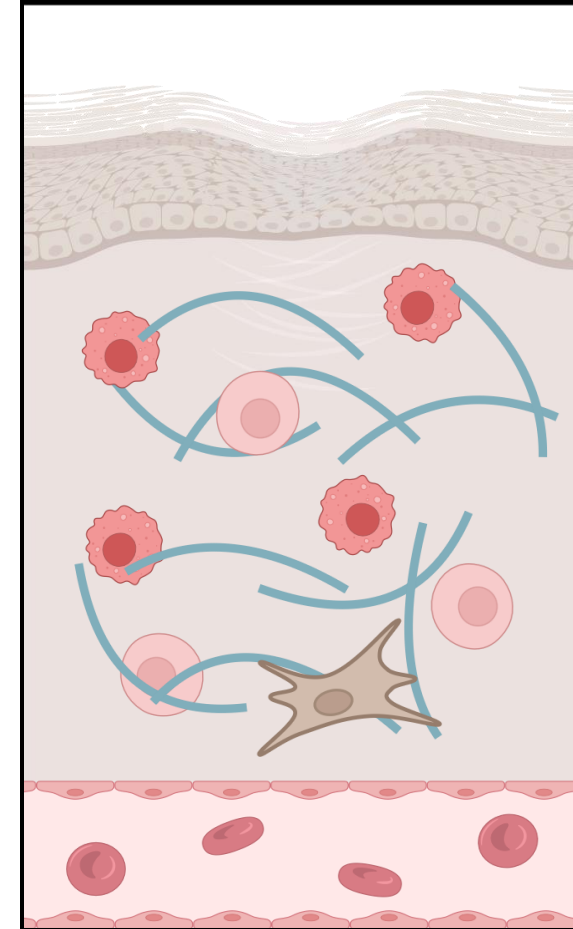
2. Inflammation



3. Proliferation



4. Remodeling



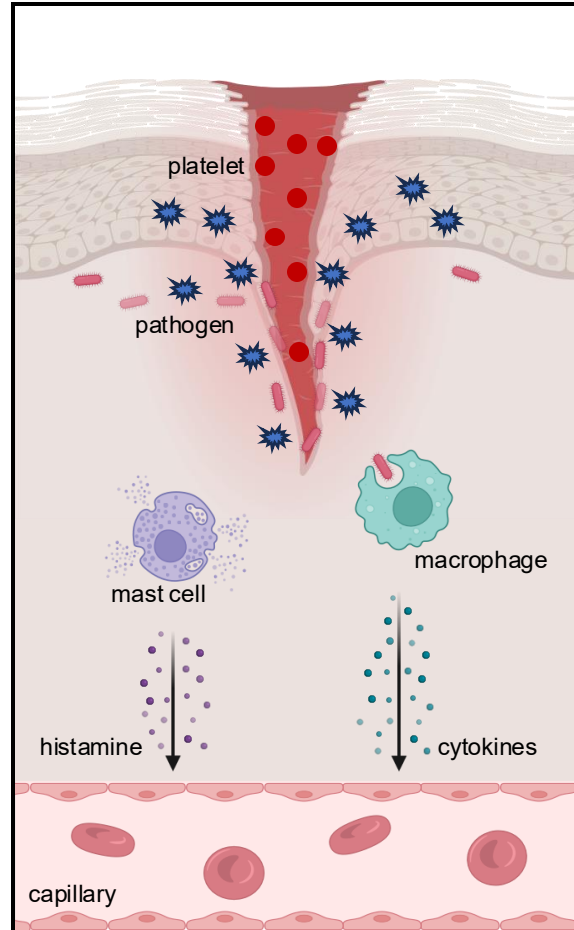
Created with BioRender.com



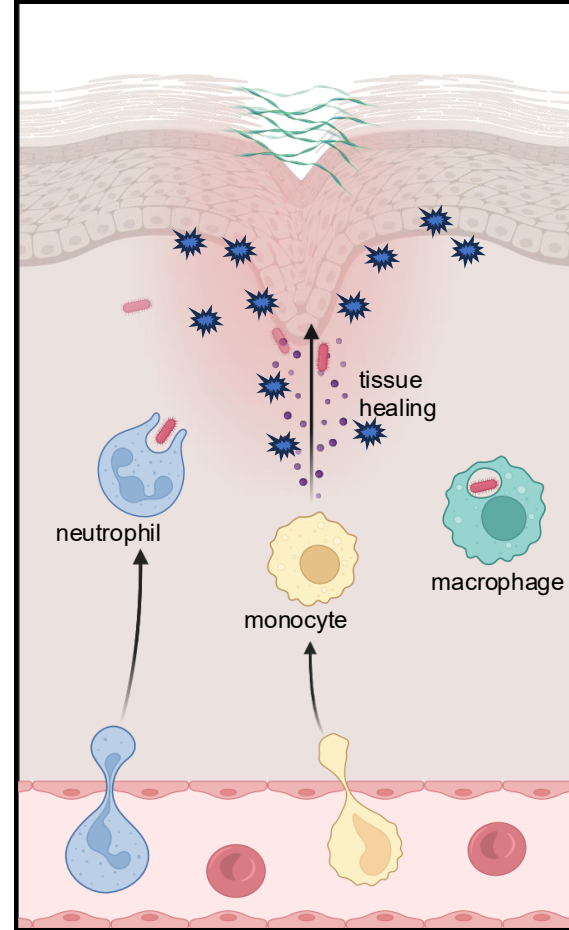
Models: Skin and Soft Tissue Wound



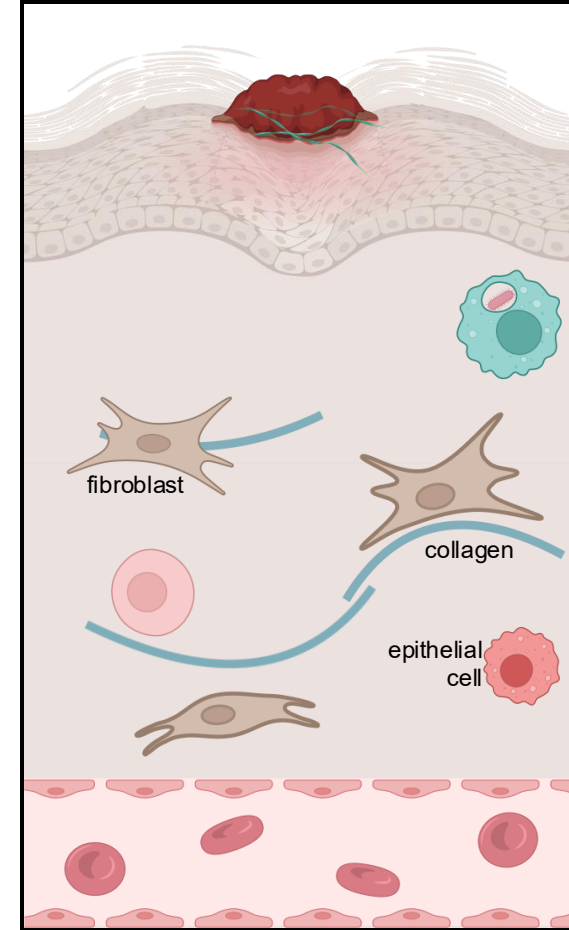
1. Hemostasis



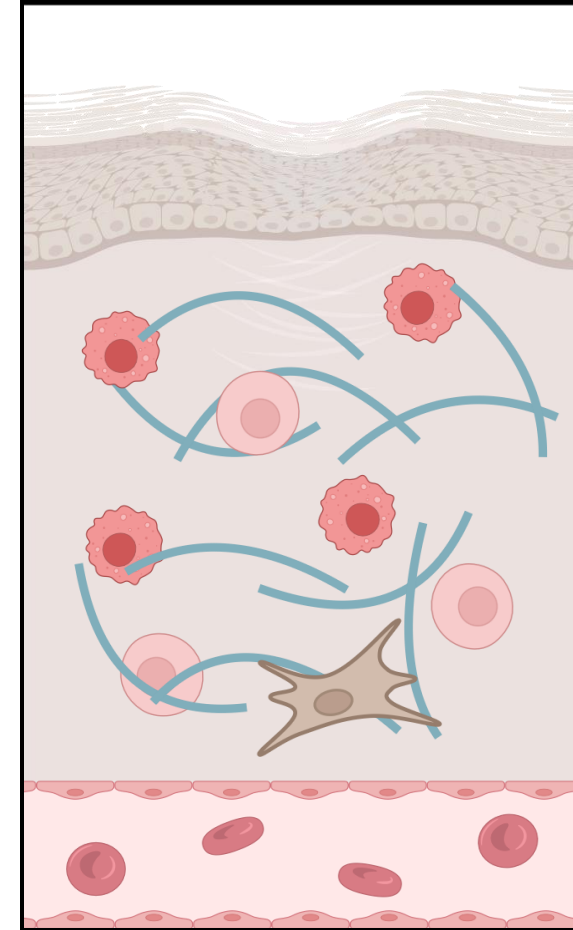
2. Inflammation



3. Proliferation



4. Remodeling



Created with BioRender.com



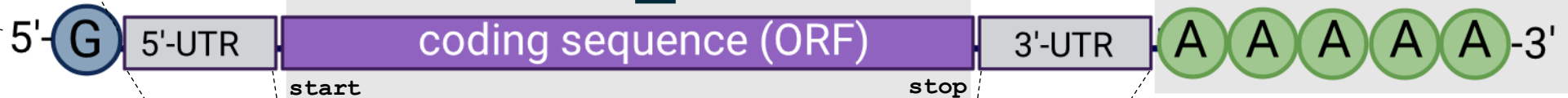
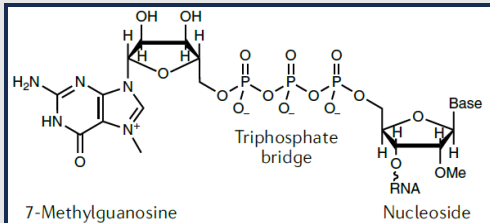
A Platform to Produce mRNA Prototypes



5' m⁷Gppp Cap

Translation initiation

Self/non-self recognition



Open Reading Frame

protein



Translation efficiency

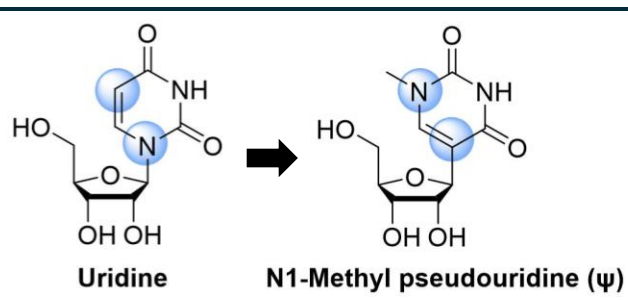
5'/3' Untranslated Regions

mRNA localization, translation and stability

PolyA Tail

Stability

Translation efficiency

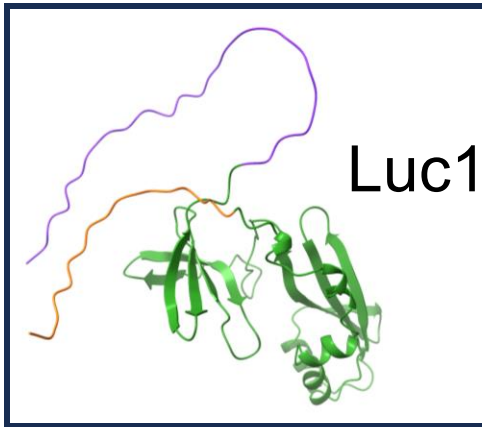
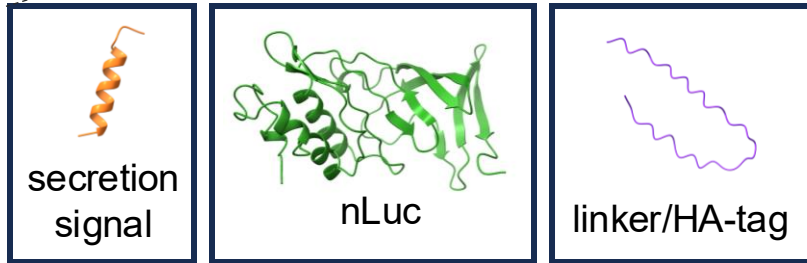
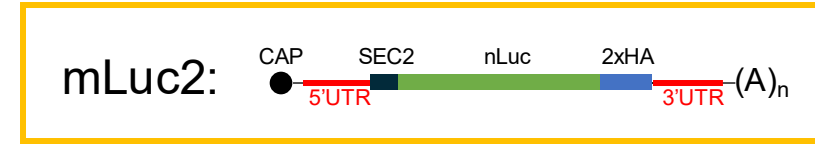




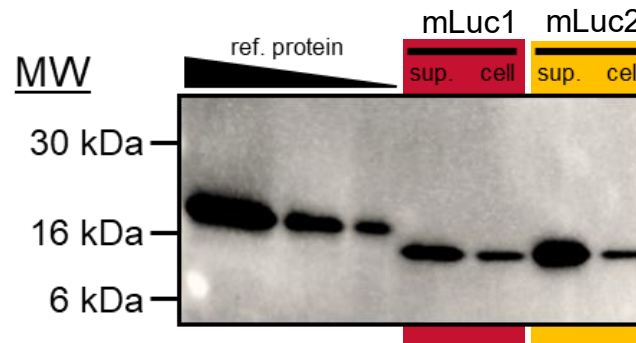
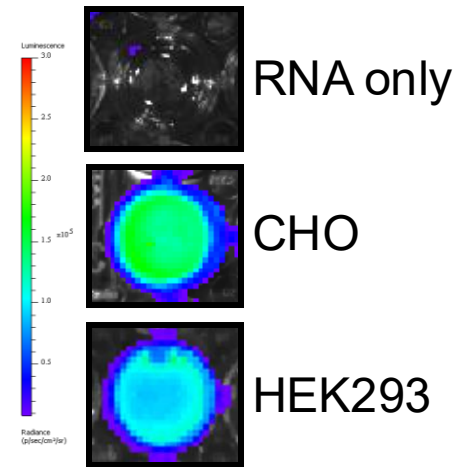
mLuc Expression in Cell Lines



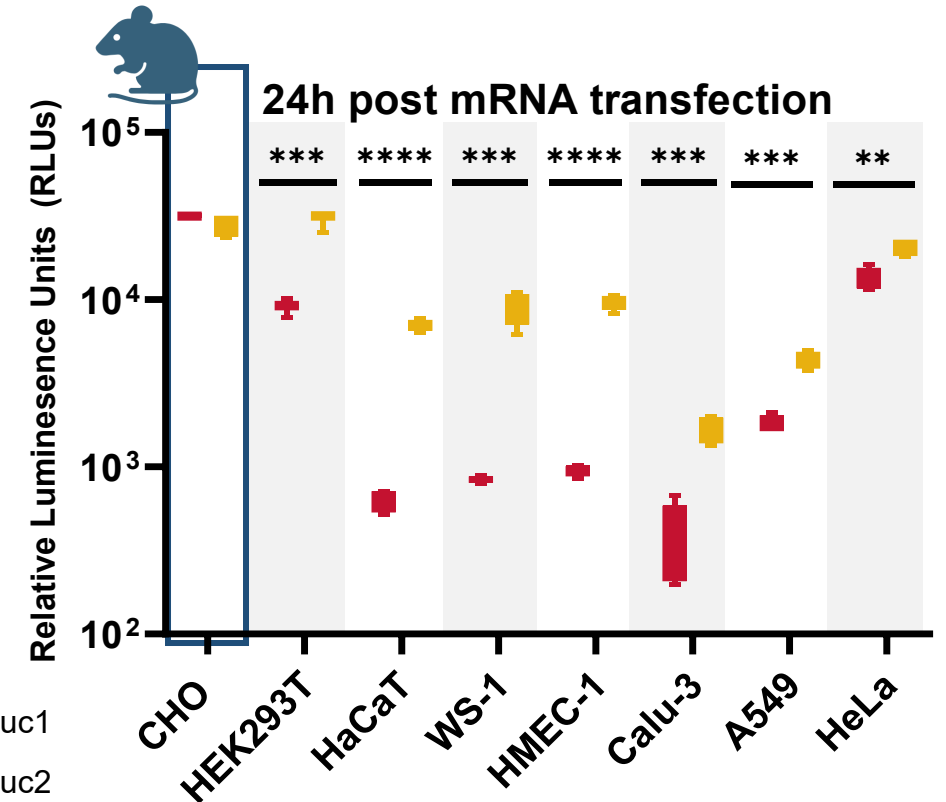
mLuc series: bioluminescence



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■ mLuc1
■ mLuc2





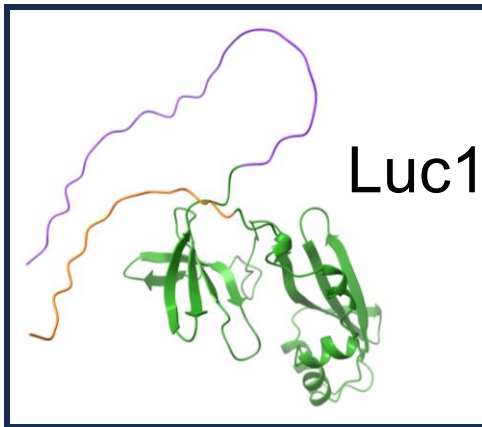
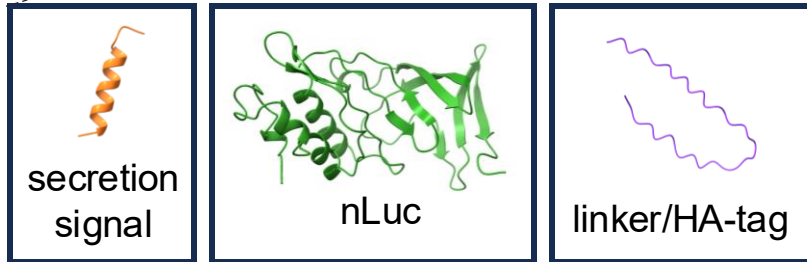
mLuc Expression in Cell Lines



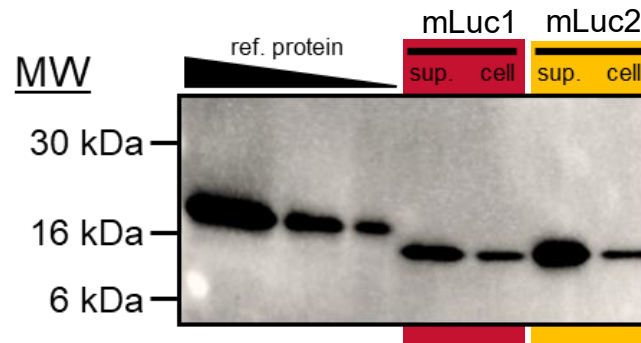
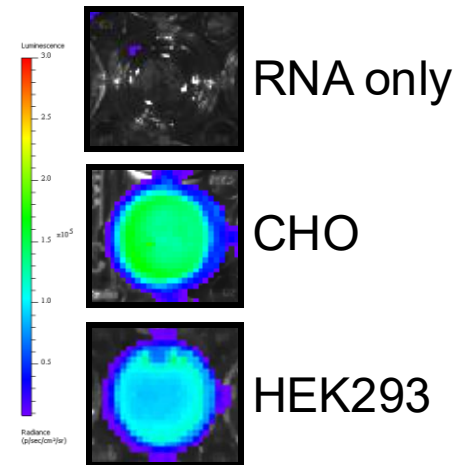
mLuc series: bioluminescence

mLuc1: CAP ● SEC1 nLuc 2xHA 3'UTR (A)_n

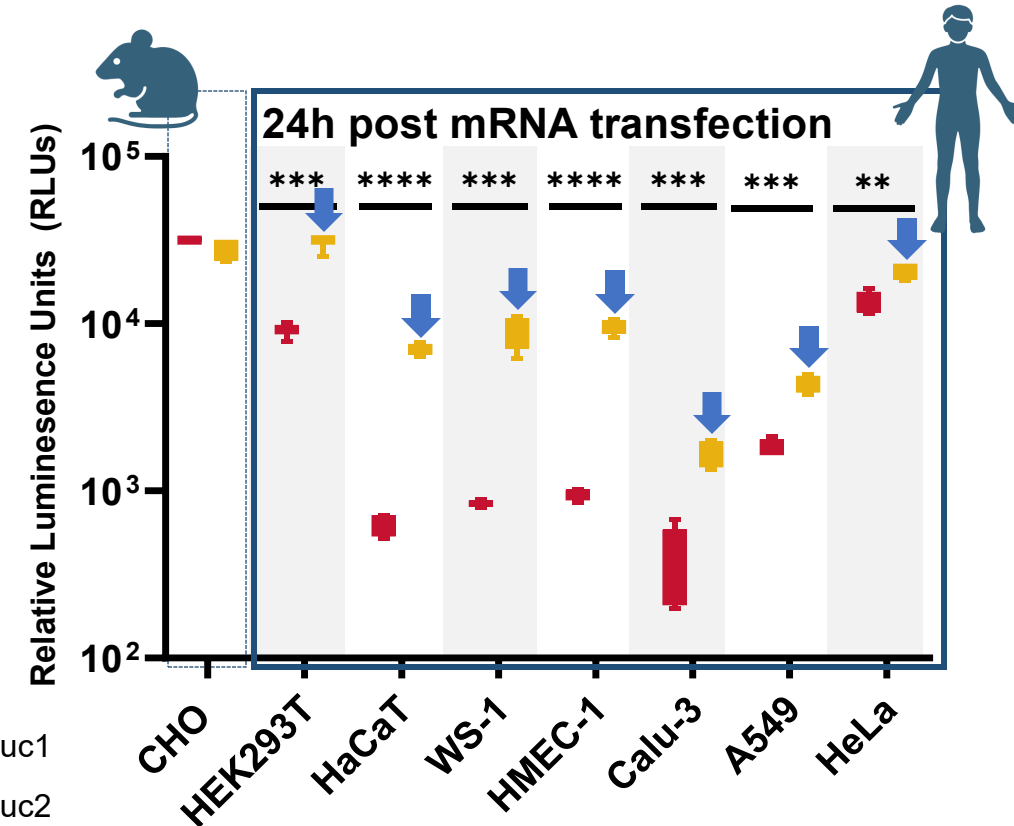
mLuc2: CAP ● SEC2 nLuc 2xHA 3'UTR (A)_n



Created with AlphaFold.com



■ mLuc1
■ mLuc2





mLuc Series Express in Murine SSTW



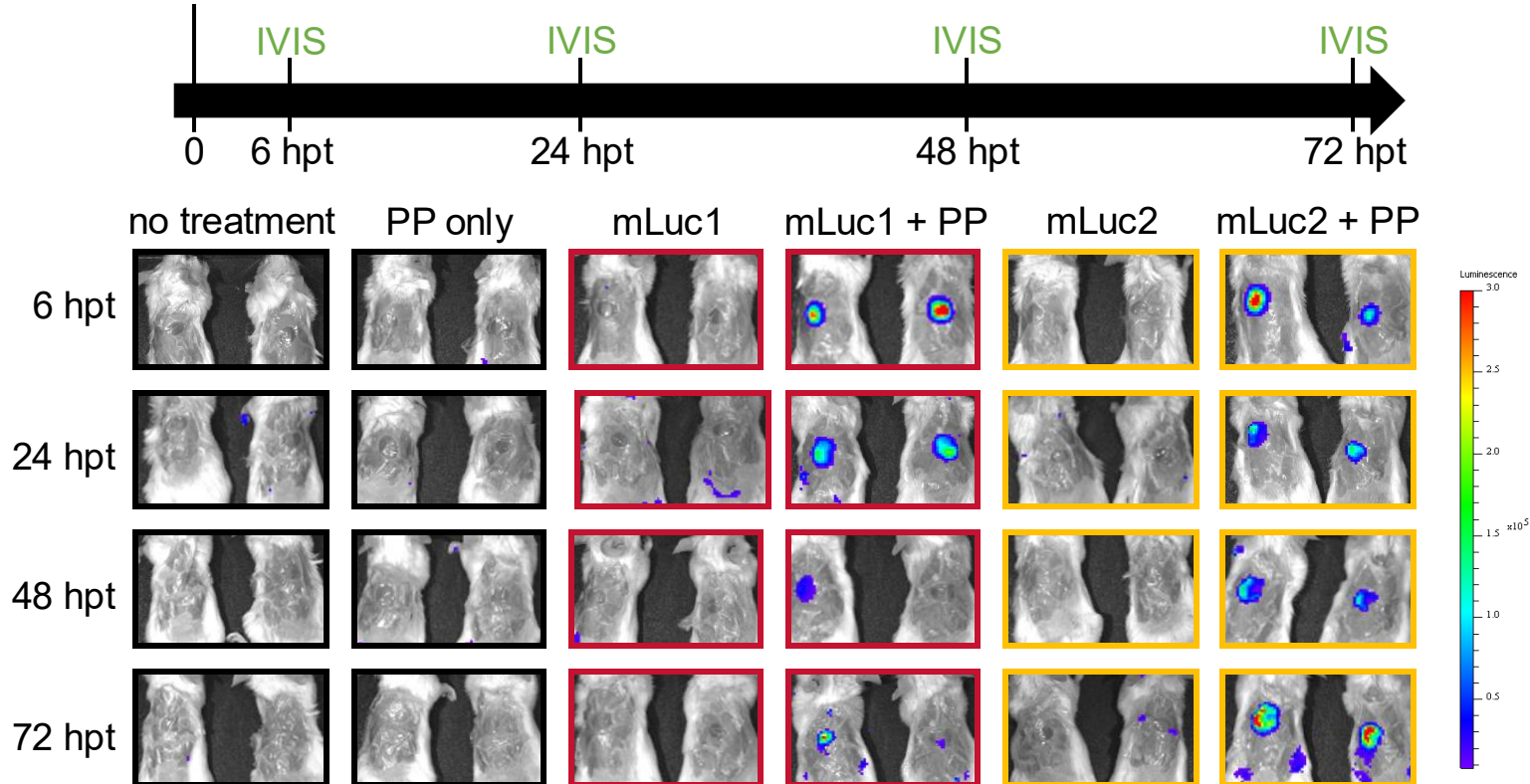
mLuc series: bioluminescence

mLuc1: CAP ● SEC1 nLuc 2xHA 3'UTR (A)_n

mLuc2: CAP ● SEC2 nLuc 2xHA 3'UTR (A)_n



- create wound (i.c. BALBc, n=10)
- apply mRNA (5 µg), +/- PP nanocarrier



*PP: Polyplus (Sartorius)



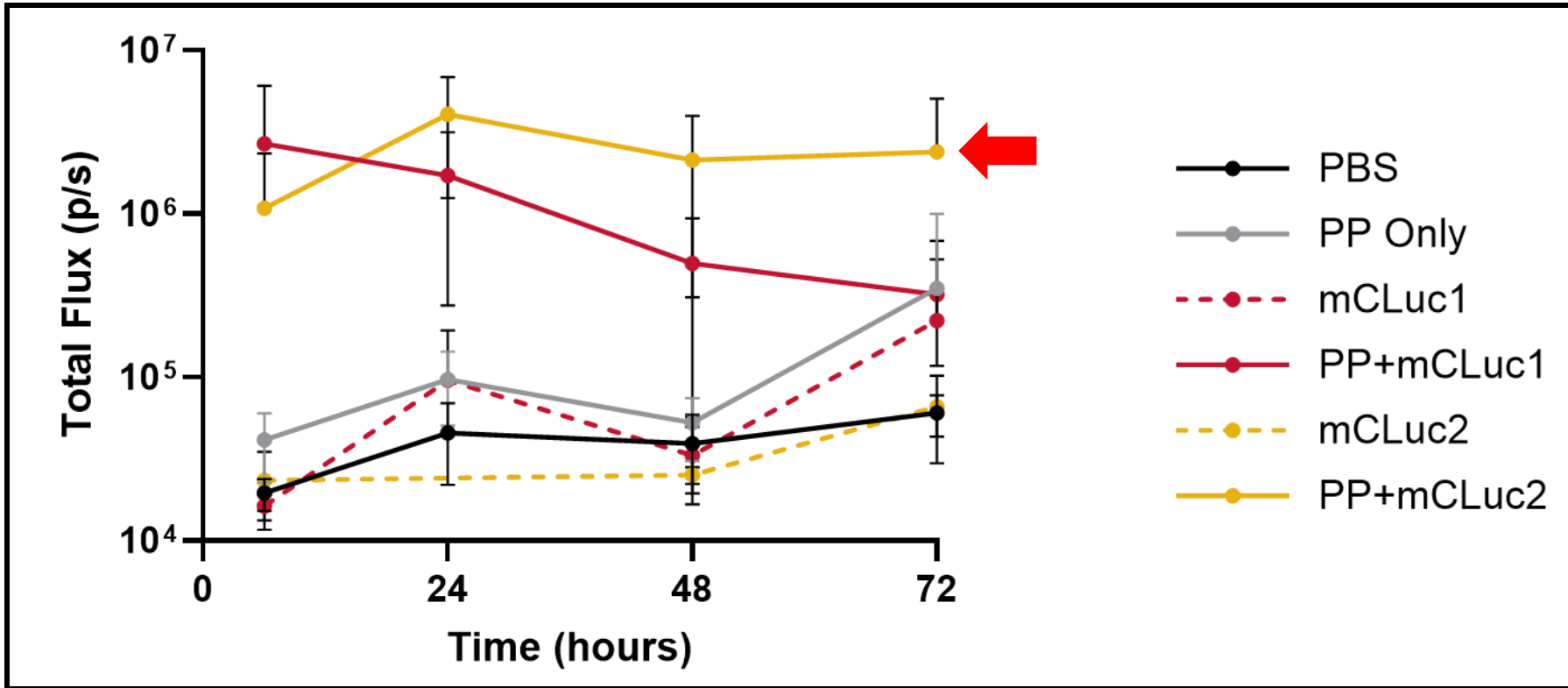
mLuc Series Express in Murine SSTW



mLuc series: bioluminescence

mLuc1: CAP ● SEC1 nLuc 2xHA 3'UTR (A)_n

mLuc2: CAP ● SEC2 nLuc 2xHA 3'UTR (A)_n



➡ mLuc2 expresses better than mLuc1 in murine SSTW beds

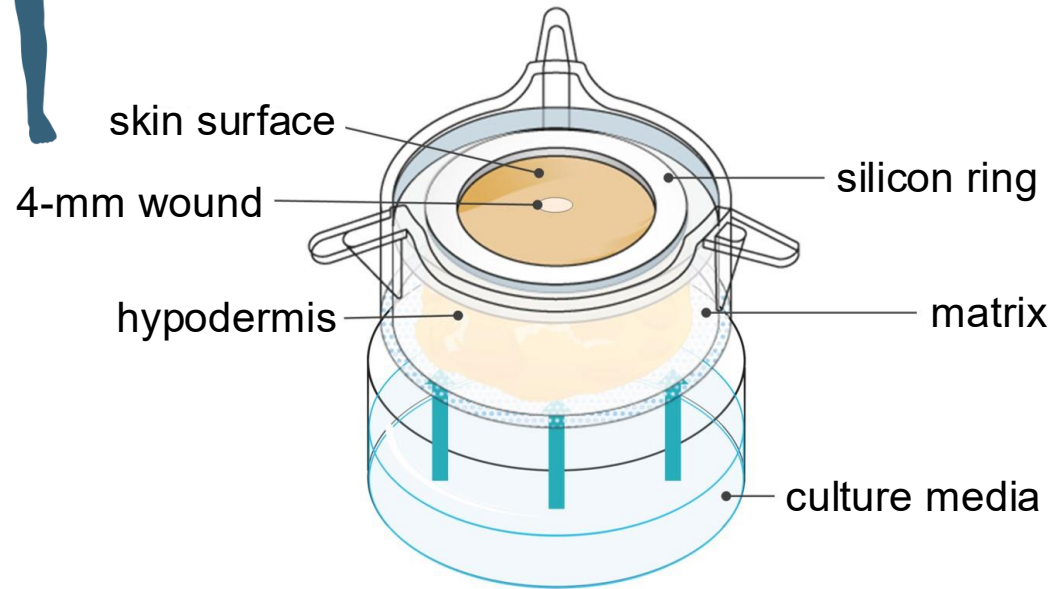
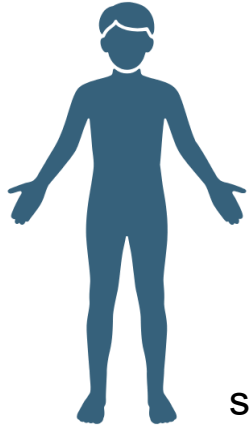


mLuc1 Expresses in Human SSTW



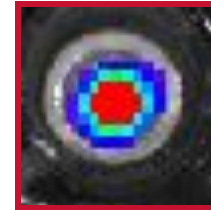
mLuc1 series: bioluminescence

mLuc1: CAP ● 5'UTR SEC1 nLuc 2xHA 3'UTR (A)_n

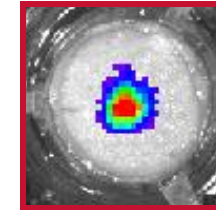


www.genoskin.com

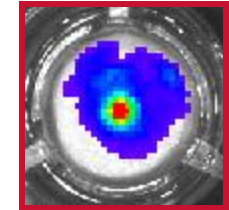
24 hpt



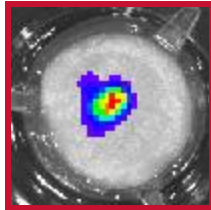
Donor 1



Donor 2



Donor 3



Donor 4



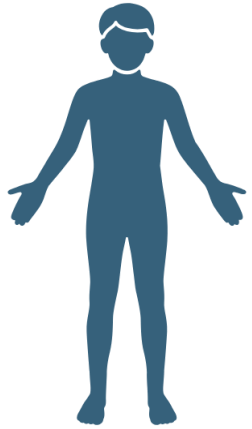
Comparing mLuc1/2 Expression



mLuc series: bioluminescence

mLuc1: CAP ● SEC1 nLuc 2xHA 3'UTR (A)_n

mLuc2: CAP ● SEC2 nLuc 2xHA 3'UTR (A)_n



- WoundSkin (4 mm), donor 1
- apply mRNA (5 µg)



PBS

PP only

mLuc1

mLuc1 + PP

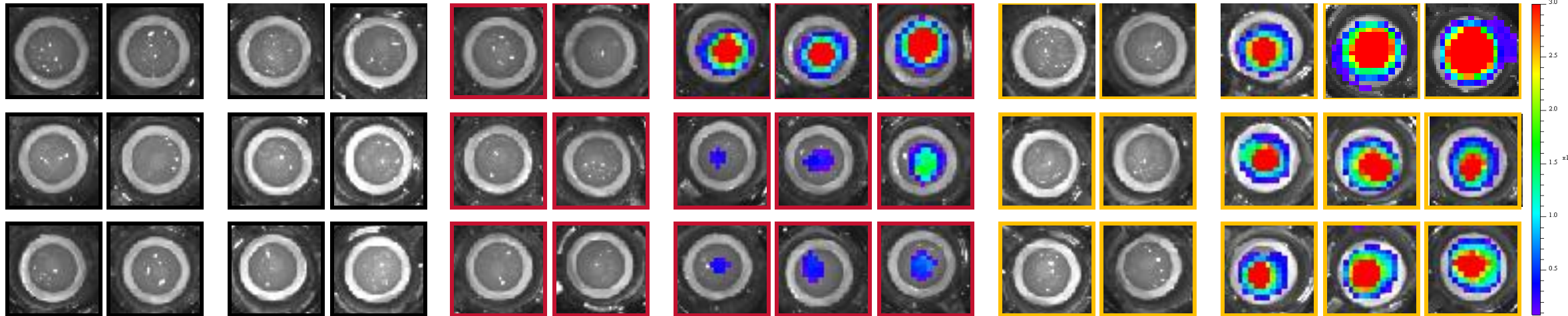
mLuc2

mLuc2 + PP

24 hpt

48 hpt

72 hpt



*PP: Polyplus (Sartorius)



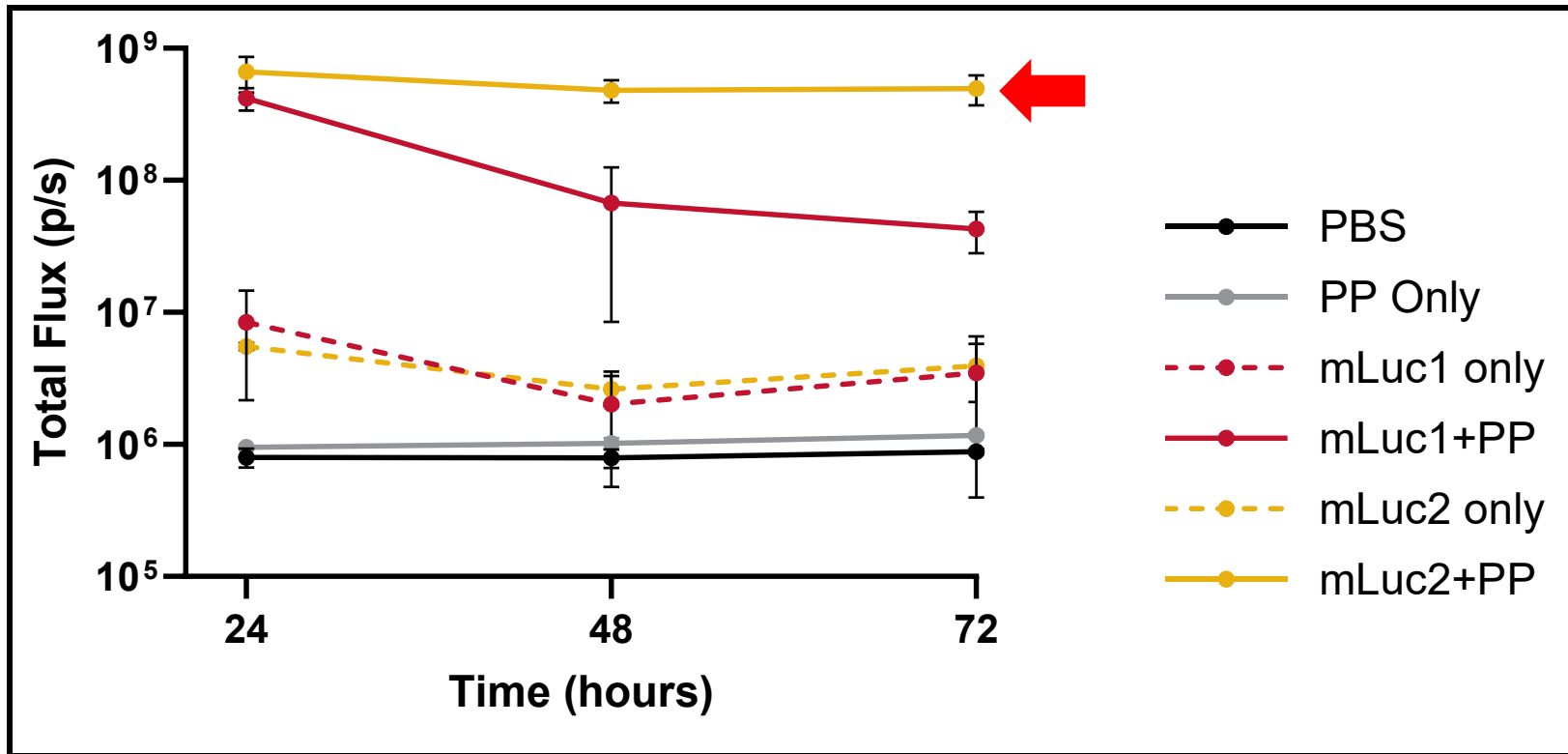
mLuc2 Expresses Better in h-SSTW



mLuc series: bioluminescence

mLuc1: CAP ● SEC1 nLuc 2xHA 3'UTR (A)_n

mLuc2: CAP ● SEC2 nLuc 2xHA 3'UTR (A)_n



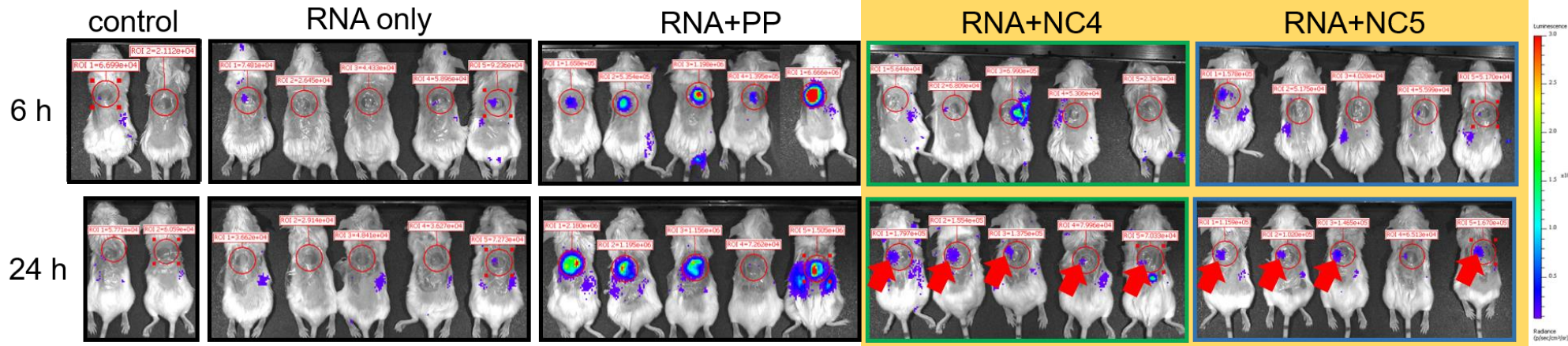
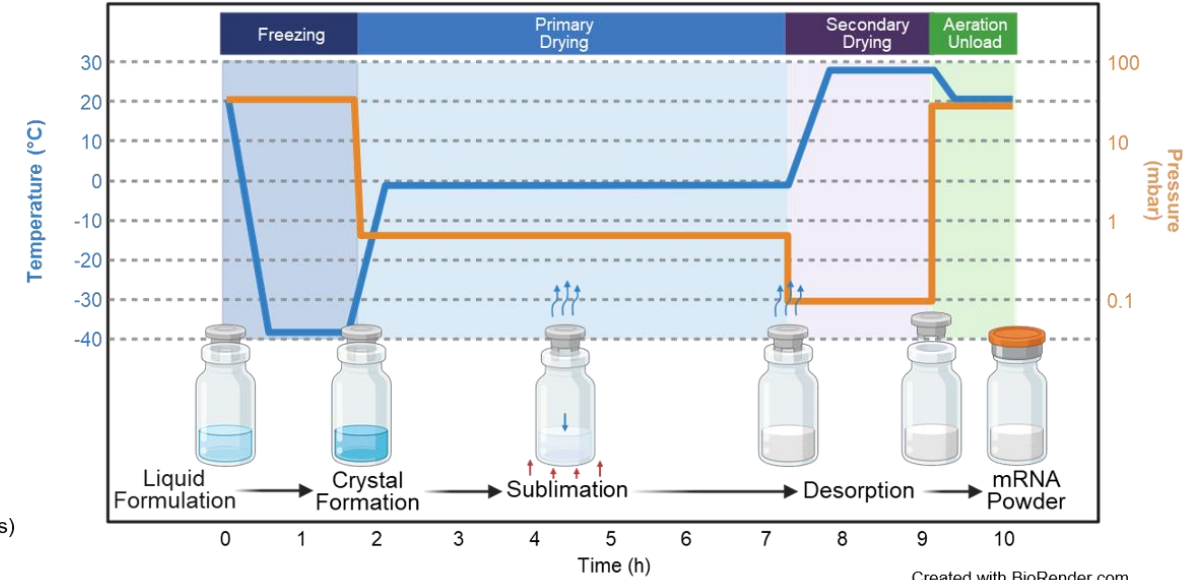
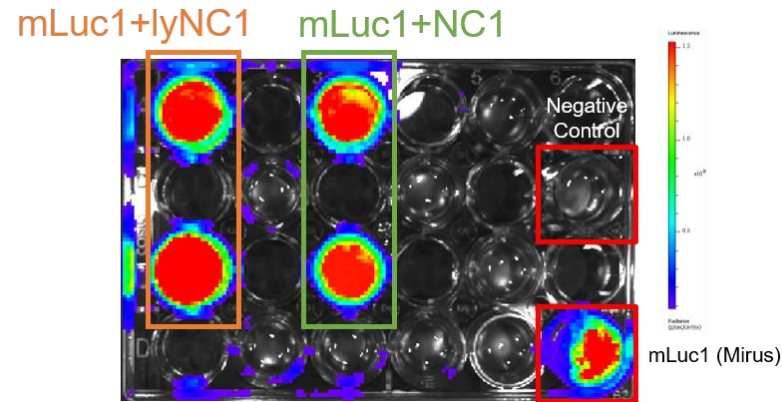
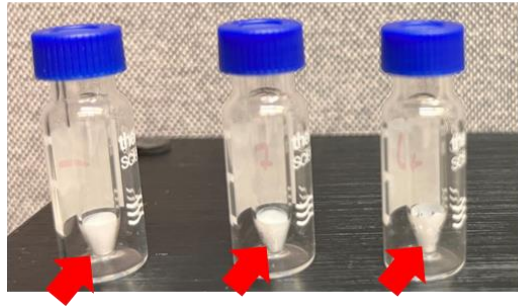
➡ mLuc2 expresses better than mLuc1 in human SSTW beds (donor 1)



Lyophilization of mRNA Products



mLuc1: CAP ● SEC1 nLuc 2xHA (A)_n
5'UTR 3'UTR





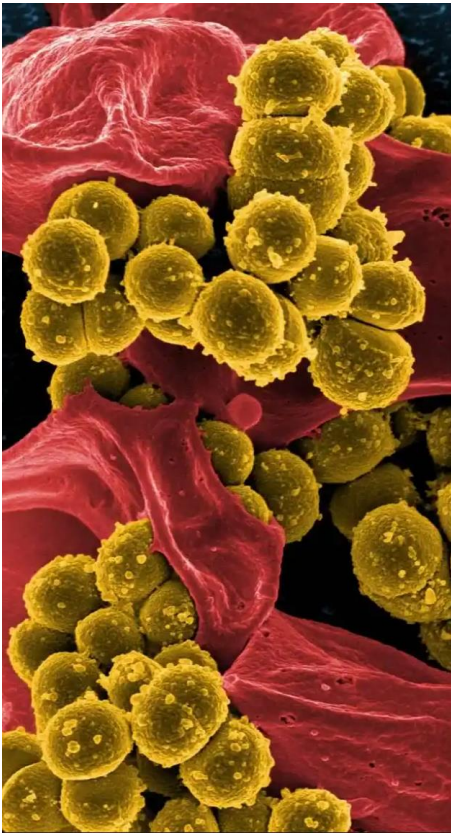
Antimicrobial mRNAs: AmRNA(s)



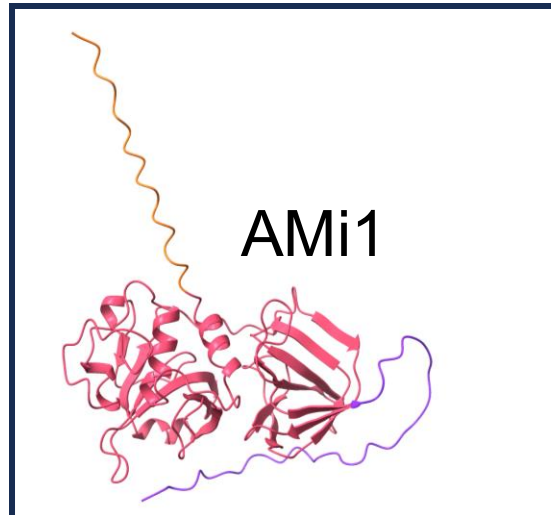
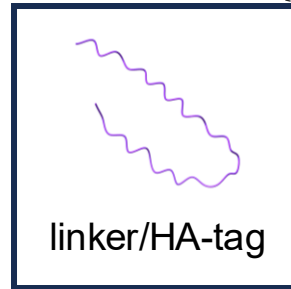
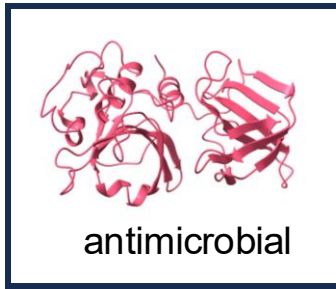
AmRNA series: antimicrobial

AmRNA1: CAP ● SEC1 5'UTR ami-1 2xHA 3'UTR (A)_n

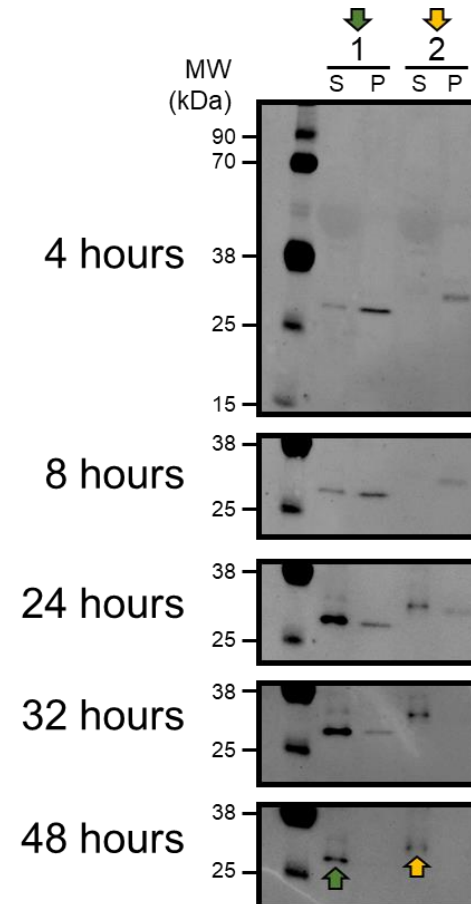
AmRNA2: CAP ● SEC2 5'UTR ami-1 2xHA 3'UTR (A)_n



www.britannica.com/science/Staphylococcus-aureus



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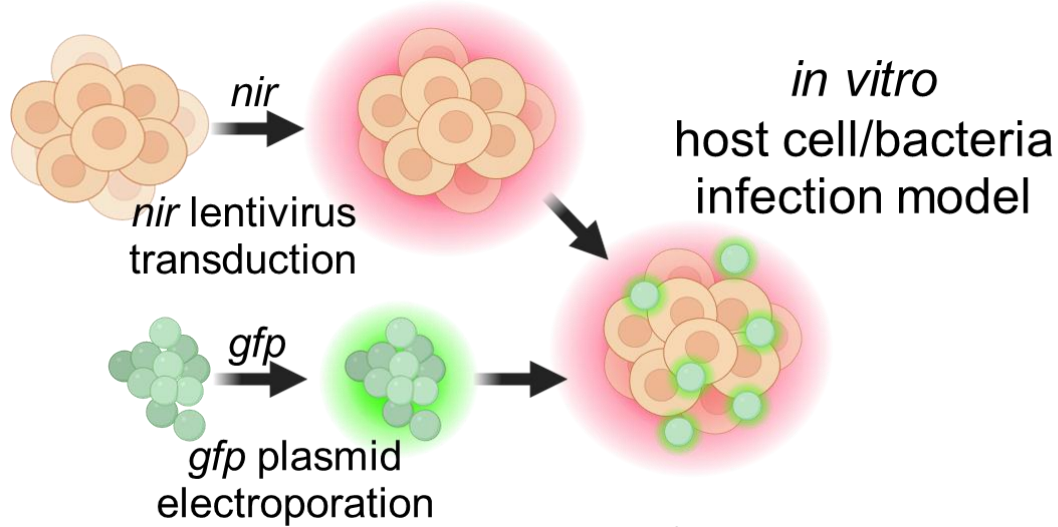
AmRNA Antimicrobial Activity



AmRNA1: CAP ● SEC1 5'UTR ami-1 2xHA 3'UTR (A)_n

AmRNA2: CAP ● SEC2 5'UTR ami-1 2xHA 3'UTR (A)_n

- High throughput pipeline to test AmRNAs: IncuCyte SX5



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- Real-time fluorescence live-cell imaging system: *S. aureus* (GFP) | host cell (NIR)

<https://www.sartorius.com/en/products/live-cell-imaging-analysis/live-cell-analysis-instruments/sx5-live-cell-analysis-instrument>



CHO Cells Infected by *S. aureus*



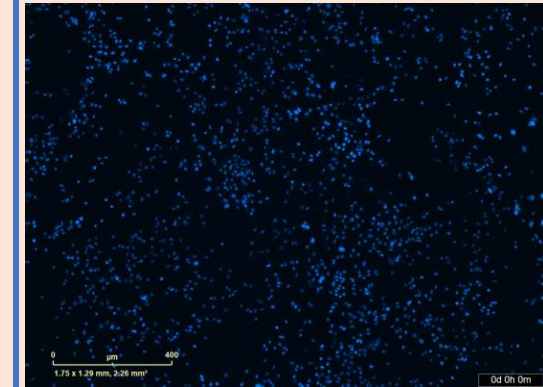
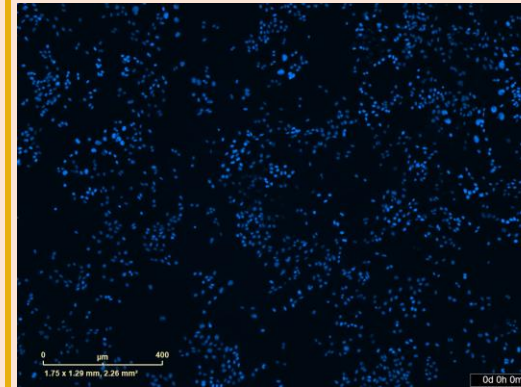
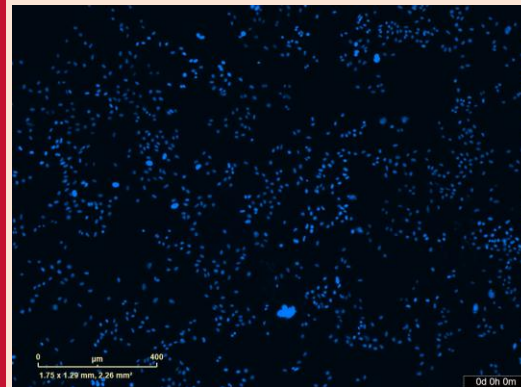
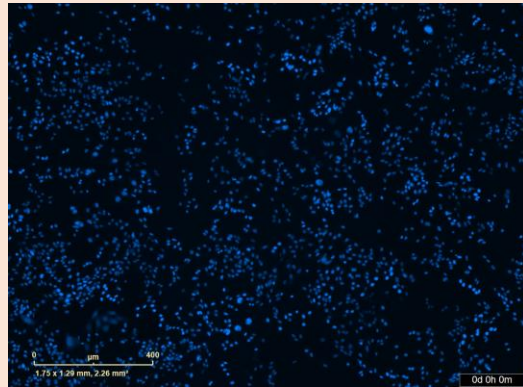
Uninfected

Infected

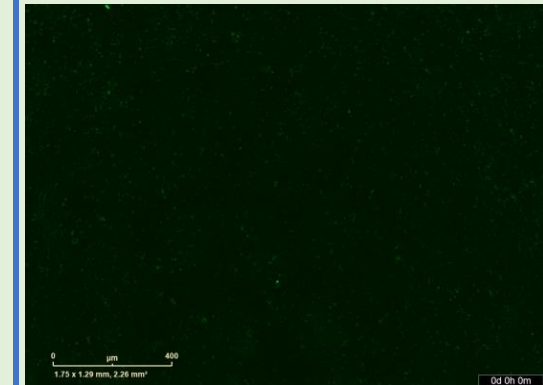
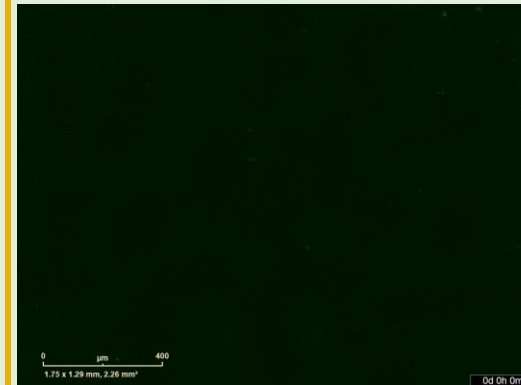
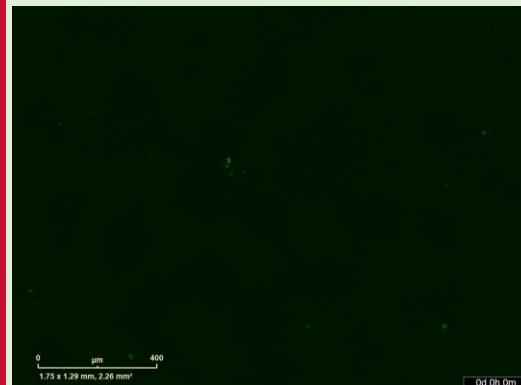
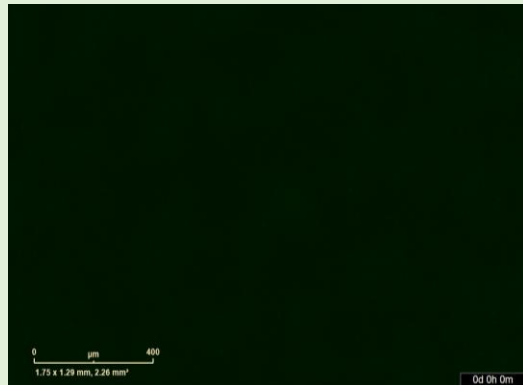
Antibiotic (Vanc)

AmRNA1

CHO cells
(NIR)



S. aureus
(GFP)





Anti-*S. aureus* Activity -CHO-



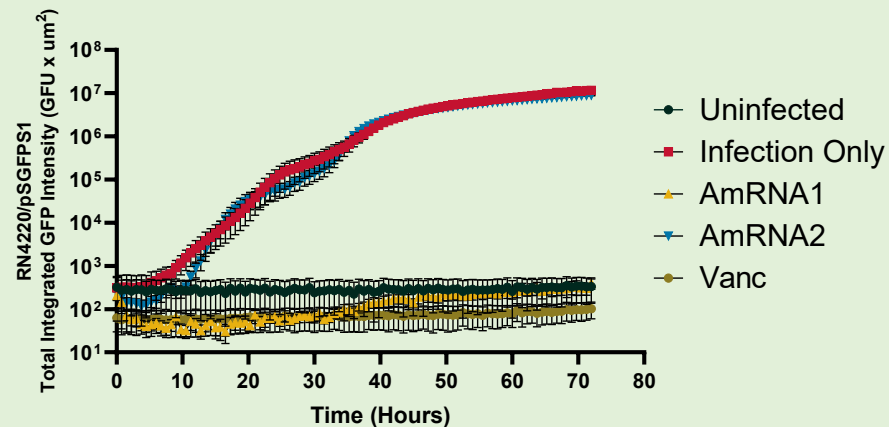
AmRNA1:



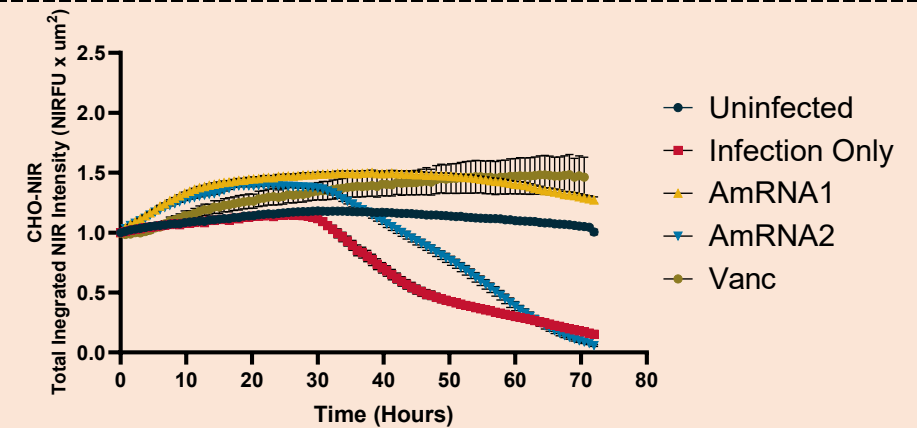
AmRNA2:



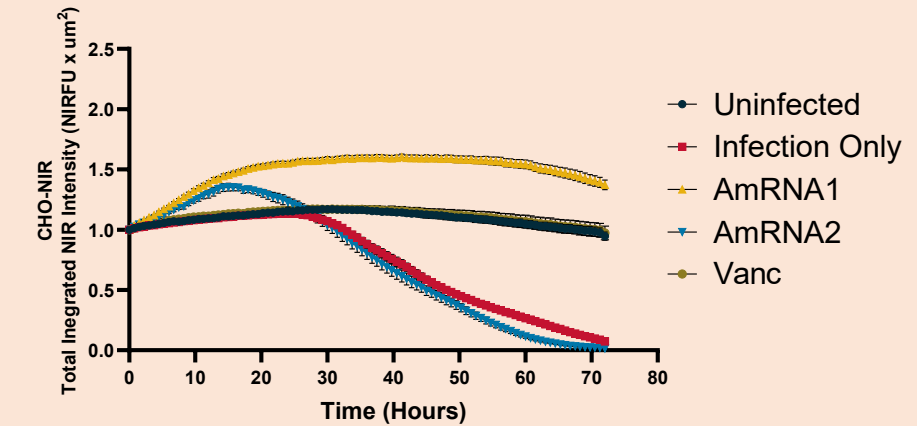
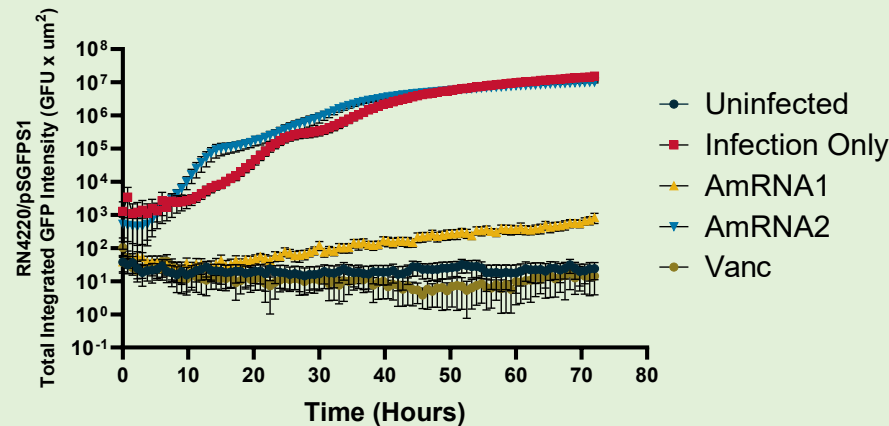
Bacterial bioburden signal over time (GFP signal)



Fate of CHO cells over time (NIR)



MOI 0.01



MOI 0.1



Activity in Human SST Cell Lines



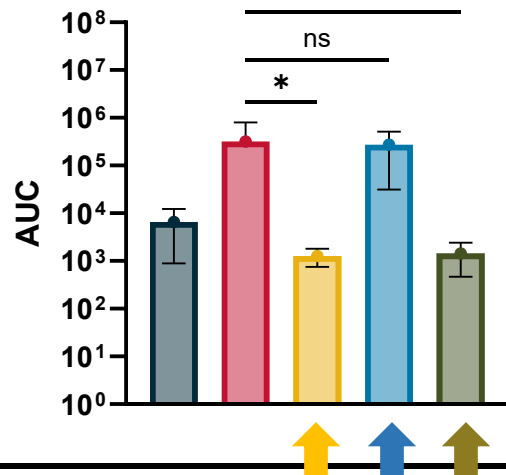
AmRNA1: CAP ● SEC1 5'UTR ami-1 2xHA 3'UTR (A)_n

AmRNA2: CAP ● SEC2 5'UTR ami-1 2xHA 3'UTR (A)_n

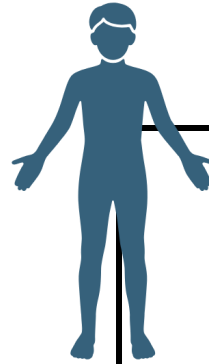
Bacterial bioburden signal over time (GFP signal), MOI 0.01



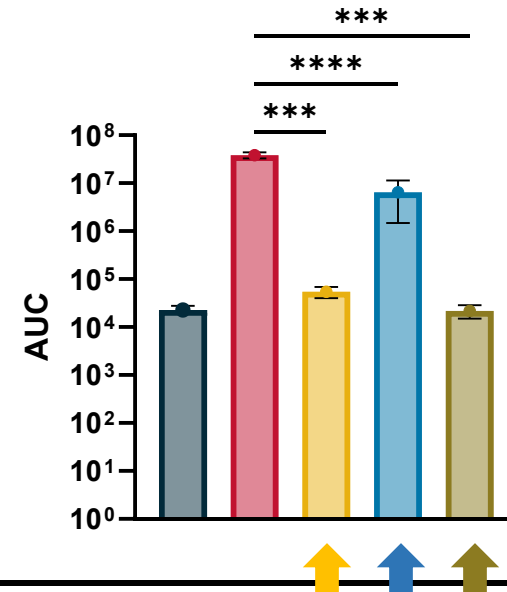
CHO cells



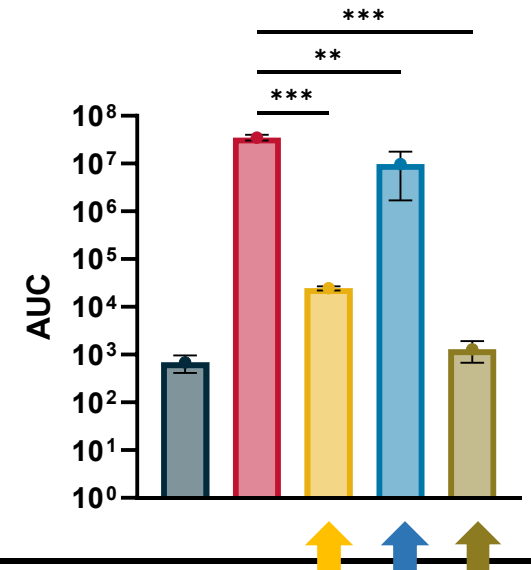
- uninfected
- infected
- ▲ AmRNA1
- ▼ AmRNA2
- Vanc



Keratinocytes (HaCat)



Fibroblasts (WS-1)





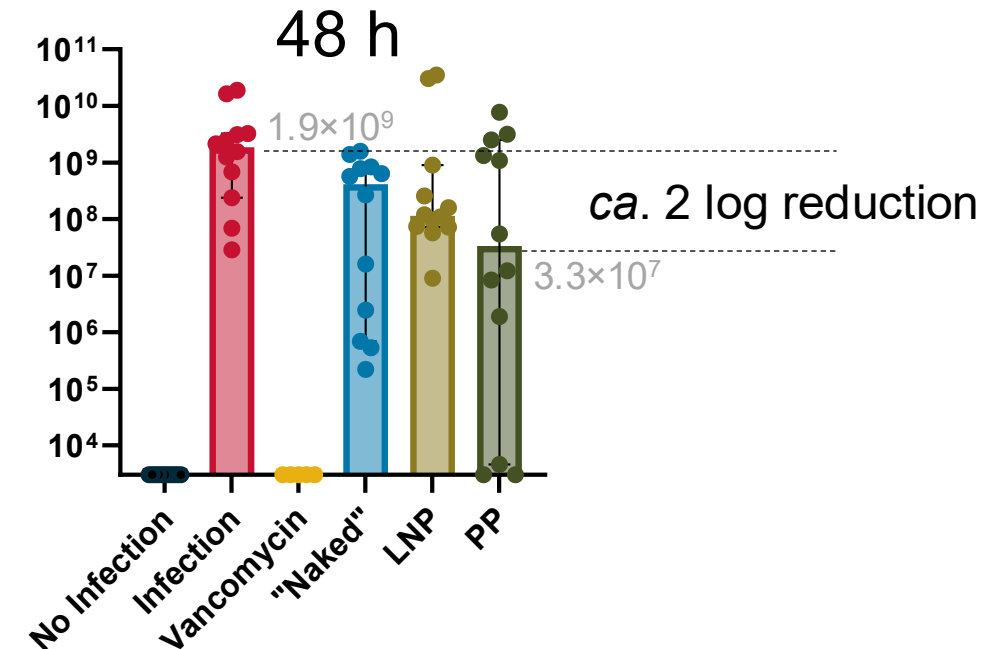
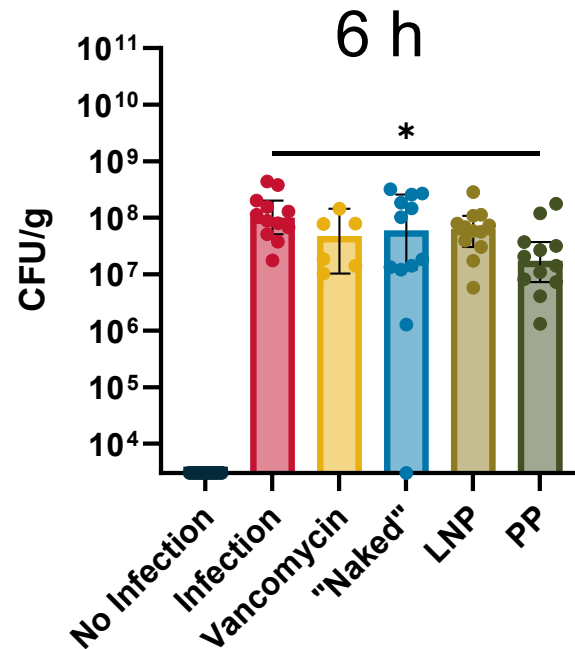
AmRNA Activity in Mouse Wound Model



AmRNA1: CAP 5'UTR SEC1 ami-1 2xHA 3'UTR (A)_n



- create wound
- apply mRNA (5 µg) (T₀)
- *S. aureus* infection (1×10⁶ CFUs) (2h)



Results presented as the median CFU/g value + 95% CI; *: p<0.01



AmRNA Antimicrobial Activity



AmRNA1: CAP ● SEC1 5'UTR ami-1 2xHA 3'UTR (A)_n

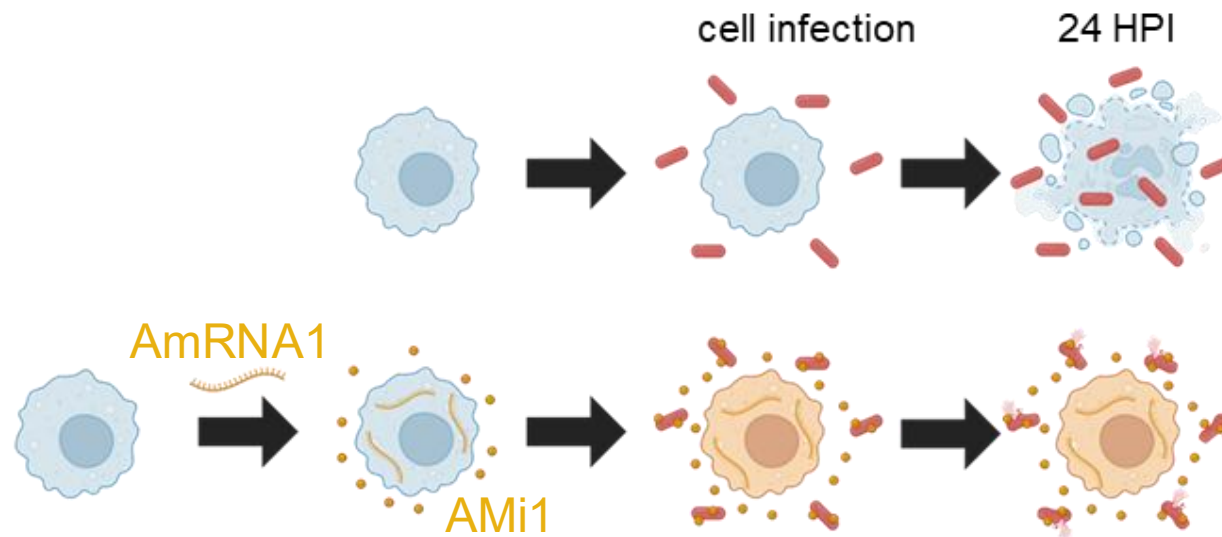


AmRNA2: CAP ● SEC2 5'UTR ami-1 2xHA 3'UTR (A)_n



mLuc1: CAP ● SEC1 5'UTR nLuc 2xHA 3'UTR (A)_n

mLuc2: CAP ● SEC2 5'UTR nLuc 2xHA 3'UTR (A)_n



➡ First *in vitro* demonstration that host cells transfected with antimicrobial-RNA can sustain *S. aureus* infection



“Building Blocks”: Modular Design



AmRNA1: CAP ● SEC1 5'UTR ami-1 2xHA 3'UTR (A)_n



AmRNA2: CAP ● SEC2 5'UTR ami-1 2xHA 3'UTR (A)_n

AmRNA3: CAP ● SEC1 5'UTR ami-1 2xHA 3'UTR (A)_n

AmRNA4: CAP ● SEC1 5'UTR ami-1 * 2xHA 3'UTR (A)_n

AmRNA5: CAP ● SEC3 5'UTR ami-1 2xHA 3'UTR (A)_n

Dr. Mackenzie Ryan: MHSRS-26-4729 (poster): Engineering Antimicrobial RNA Therapeutics to Combat *S. aureus* in Human Cell Models of Combat Wound Infections.



“Building Blocks”: Modular Design



AmRNA1: CAP ● SEC1 5'UTR ami-1 2xHA 3'UTR (A)_n



AmRNA2: CAP ● SEC2 5'UTR ami-1 2xHA 3'UTR (A)_n



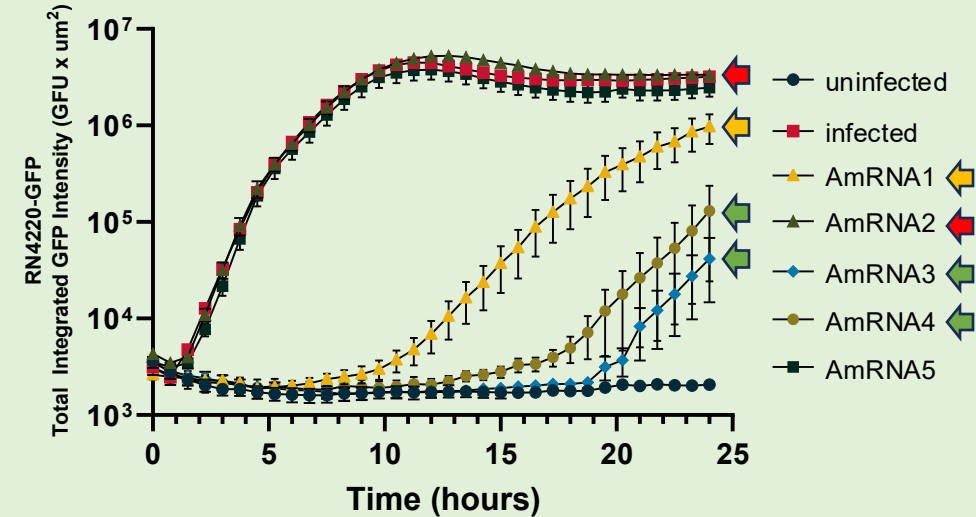
AmRNA3: CAP ● SEC1 5'UTR ami-1 2xHA 3'UTR (A)_n



AmRNA4: CAP ● SEC1 5'UTR ami-1 * 2xHA 3'UTR (A)_n

AmRNA5: CAP ● SEC3 5'UTR ami-1 2xHA 3'UTR (A)_n

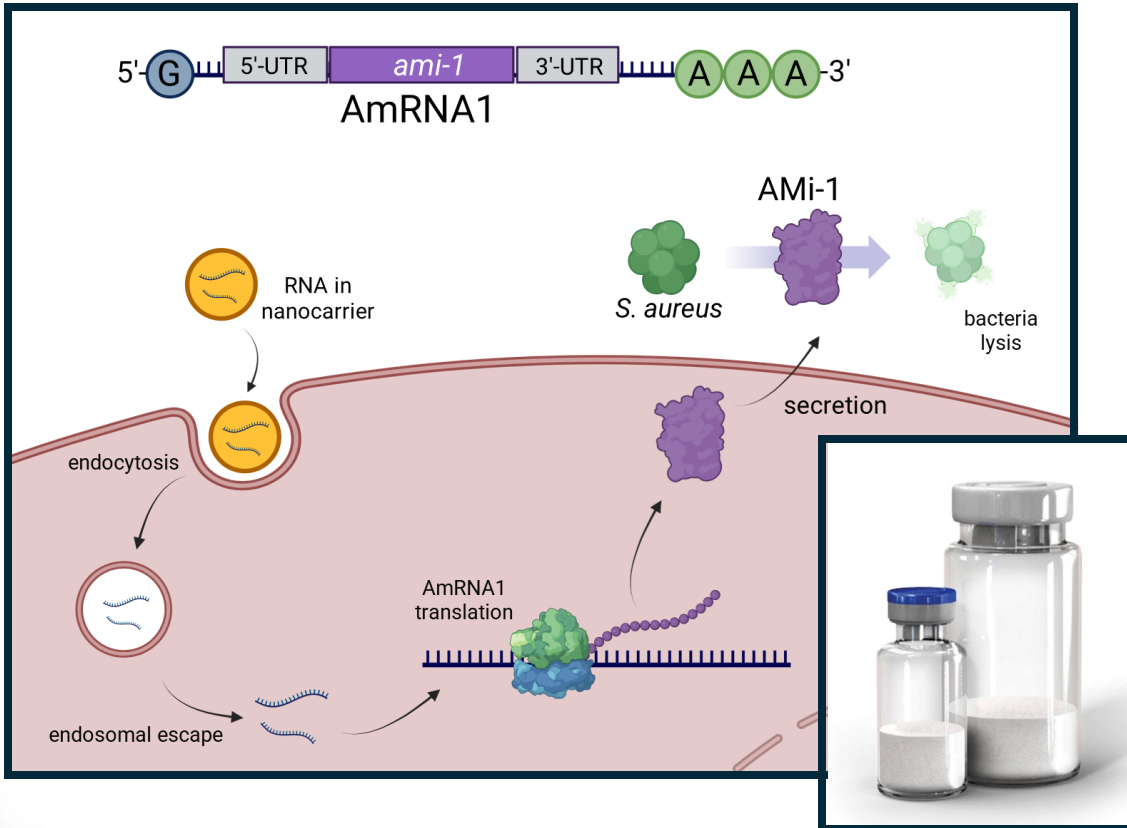
Bacterial bioburden (GFP signal) | HaCaT | MOI 1



Dr. Mackenzie Ryan: MHSRS-26-4729 (poster): Engineering Antimicrobial RNA Therapeutics to Combat *S. aureus* in Human Cell Models of Combat Wound Infections.



Summary



- Joint Navy/Army platform for mRNA production
- AmRNA: new approach to develop antimicrobials
- AmRNAs: non-toxic for the host
- Lyophilized AmRNA products: austere environments
- Modular AmRNA: optimization for human systems

Antimicrobial Compositions and Methods, U.S. Appl. No. 63/845,799 and 64/042,035.

Inventors: Yoann Le Breton; Evelina Angov.

Assignee: The United States of America as Represented by the Secretary of the Navy Washington, DC (US).



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Thank You For Your Attention

Monoclonal Antibodies



Dr. Lucas Brown: MHSRS-26-5513 (poster): Evaluation of Field Deployable Broadly Neutralizing Monoclonal Antibodies to Counter MRSA Infections in Combat Wounds.

Bacteriophages

Anti-Biofilm Agents

Dr. John Vitucci: MHSRS-26-3821 (poster): Efficacy of a Novel Antimicrobial Peptide in Eradicating Multidrug-Resistant Bacterial Infections and Biofilms in Preclinical Mouse and Porcine Models.

**Antimicrobial
Proteins/Peptides**

Enhanced Dressings

mRNA Medicine

Dr. Zachary Rivas: MHSRS-26-6075 (talk): Advancement of Pathogen-Agnostic CLAROMER® Peptoids as Deployable Countermeasures for Battlefield Wound Infection Treatment.

Dr. Mackenzie Ryan: MHSRS-26-4729 (poster): Engineering Antimicrobial RNA Therapeutics to Combat *Staphylococcus aureus* in Human Cell Models of Combat Wound Infections.

Dr. Corley Bernstein: MHSRS-26-3702 (poster): Illuminating *Klebsiella pneumoniae* Infections: A Bioluminescent Platform for Longitudinal Assessment of Antibacterial Efficacy



READINESS THROUGH RESEARCH & DEVELOPMENT